

WE CLAIM:

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1. A homogeneous assay method for detecting the presence of a target polynucleotide in a sample comprising the steps of:

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- a) Contacting said target polynucleotide with a single-stranded polynucleotide probe, said polynucleotide probe comprising a polynucleotide and at least a first entity and a second entity, wherein said first entity is attached to a first nucleotide of said polynucleotide by means of a first linker arm, and said second entity is attached to a second nucleotide by means of a second linker arm, said first and said second nucleotides separated by a stretch of about ten other nucleotides, said entities comprising a characteristic, wherein upon the hybridization per se of said polynucleotide probe to said target polynucleotide, said characteristic enables the generation of a change in a property either in the polynucleotide probe, in the target polynucleotide, or in both, wherein said property change is the signal, provided that said property change will not be substantially generated when said polynucleotide probe is not hybridized to said target;

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- b) forming a hybrid comprising said polynucleotide probe and said target polynucleotide; and

c) detecting the presence of said target polynucleotide by means of said property change.

- 5 2. The method of Claim 1 wherein said target polynucleotide is in double-stranded form.
3. The method of Claim 1 further comprising the step of rendering said target polynucleotide in substantially single-stranded form prior to said hybrid-forming step.
- 10 4. The method of Claim 1 wherein said characteristic is the ability of said entities to intercalate into said hybrid.
- 15 5. The method of Claim 4 wherein said property change is a shift in the radiation energy of said entities.
- 20 6. The method of Claims 5 wherein said radiation is fluorescence emission.
- 25 7. The method of Claim 4 wherein said property change is an increase in the thermodynamic stability of said hybrid.
- 30 8. The method of Claim 4 wherein said entities are aromatic dyes.
9. The method of Claim 8 wherein said dyes are selected from the group consisting of phenanthridines, acridines, and anthracenes.
- 35 10. The method of Claim 9 wherein said phenanthridine is selected from the group consisting of ethidium,

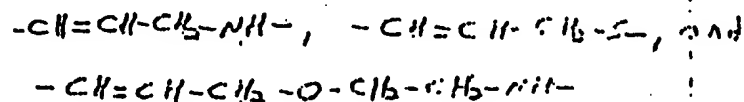
propidium, dimidium, and phenidium.

- 5 11. The method of Claim 1 wherein said linker arms are attached to base moieties of said polynucleotide probe.
- 10 12. The method of Claim 11 wherein said base is selected from the group comprising purines, pyrimidines and deazapurines.
- 15 13. The method of Claim 12 wherein said purine is adenine or guanine, and wherein said linker arm is attached at the 8 or exocyclic 6-amino position when said purine is adenine, or at the 8-position when said purine is guanine.
- 20 14. The method of claim 13 wherein said linker arm is attached at the 8-position.
- 25 15. The method of Claim 12 wherein said base is a deazapurine, and wherein said linker arm is attached at the 7 or 8-position.
- 30 16. The method of Claim 15 wherein said linker arm is attached at the 7-position.
- 35 17. The method of Claim 12 where said pyrimidine is uracil or cytosine, and wherein said linker arm is attached at the 5 or 6-position when said pyrimidine is uracil, or at the 5,6, or exocyclic 4-amino position when said pyrimidine is cytosine.
18. The method of Claim 17 wherein said linker arm is attached at the 5-position.
19. The method of Claim 11 wherein said linker arm

comprises at least 3 carbon atoms.

20. The method of Claim 19 wherein said linker arm comprises a double bond at an alpha position relative to the base.

21. The method of Claim 20 wherein said linker arm is selected from the group consisting of



22. The method of Claim 21 wherein said linker arm is allylamine ($\text{C}=\text{C}-\text{C}-\text{NH}_2$).

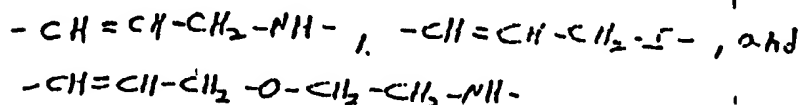
23. The method of Claim 22 wherein said allylamine is attached to the 5-position of uracil.

24. The method of Claim 11 wherein said linker arm attached to said base moiety in one fragment.

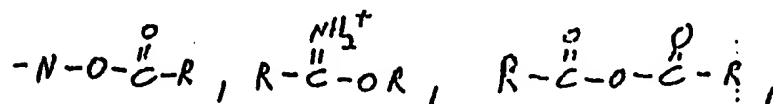
25. The method of Claim 24 wherein said fragment contains a functional group selected from the group consisting of $-\text{S}-$, $-\text{C}-\text{O}-$, $-\text{O}-$

26. The method of Claim 11 wherein said linker arm is synthesized by first attaching a first fragment to said base moiety and then attaching a second fragment to said first fragment.

27. The method of Claim 26 wherein said first fragment consists of the group selected from



and said second fragment consists of the group selected from



- 5 28. The method of Claim 10 wherein said propidium is
5-(4'-thiobutyl)-3,8-diamino-6-phenylphenanthridine.
- 10 29. The method of Claim 28 wherein said 5-(4'-
thiobutyl)-3,8-diamino-6-phenylphenanthridine is
attached via an allyamine linker arm to the
5-position of uracil.
- 15 30. The method of Claim 1 wherein said linker arm is
attached to a sugar moiety at the 1-position when
the sugar is deoxyribose or to the 1 or 2 position
when the sugar is ribose.
- 20 31. The method of Claim 28 wherein said linker arm is
attached at the 1-position, and wherein said linker
arm comprises the functionality selected from the
group consisting of amines, hydrazines, and
hydrazides.
- 25 32. The method of Claim 31 wherein said functionality
is hydrazine or hydrazide.
- 30 33. The method of Claim 1 wherein said linker arm is
attached to a phosphate moiety.
- 35 34. The method of Claim 33 wherein said polynucleotide
probe comprises about one entity per about four
nucleotides.
- 35 35. The method of Claim 1 wherein said polynucleotide
portion of said polynucleotide probe comprises a
sequence of about twelve bases.

36. The method of Claim 1 wherein one of said entities is attached to a terminal nucleotide.

5 37. The method of Claim 8 wherein said aromatic dyes are attached covalently to said linker arms.

10 38. The method of Claim 8 wherein at least one of said aromatic dyes is attached non-covalently to one of said linker arms.

15 39. The method of Claim 38 wherein said aromatic dye comprises a first chelating agent, wherein said linker arm comprises a second chelating agent, and wherein said aromatic dye is attached non-covalently to said linker arm by means of a transition metal complexed to said first and said second chelating agents.

20 40. The method of Claim 39 wherein said first or second chelating agent is selected from the group consisting of ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, and trans-diaminocyclohexanetetraacetic acid.

25 41. The method of Claim 39 wherein said transition metal is a lanthanide metal.

30 42. The method of Claim 1 wherein said polynucleotide probe comprises a first polynucleotide and at least a first entity and a second entity, wherein said first entity is attached to a first nucleotide of said first polynucleotide by means of a first linker arm and said second entity is attached to a second nucleotide of said first polynucleotide by means of a second linker arm, and a second polynucleotide and at least a third

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and fourth entity, wherein said third entity is attached to a first nucleotide of said second polynucleotide by means of a third linker arm and said fourth entity is attached to a second nucleotide of said second polynucleotide by means of a fourth linker arm, wherein said first and said second polynucleotides are complementary to adjacent, nonoverlapping base sequences of said target polynucleotide, and wherein the combination of said first, second, third and fourth entities upon the hybridization per se of said first and said second polynucleotides to said target polynucleotide results in the generation of said property change.

43. A method for preventing the in-vivo transcription of a target double-stranded polynucleotide or the in-vivo translation of a target single-stranded polynucleotide comprising the steps of:

- (a) Contacting said target polynucleotide with a single-stranded polynucleotide drug, said polynucleotide drug comprising a polynucleotide and at least a first entity and a second entity, wherein said first entity is attached to a first nucleotide of said polynucleotide by means of a first linker arm, and said second entity is attached to a second nucleotide by means of a second linker arm, said first and said second nucleotides separated by a stretch of about ten other nucleotides, said entities comprising a characteristic, wherein upon the hybridization of said polynucleotide probe to said target polynucleotide, said

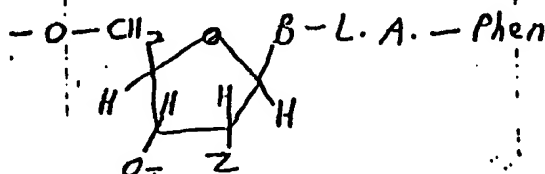
characteristic enables the generation of a change in a property either in the polynucleotide drug, in the target polynucleotide, or in both, said provided that said property change will not be substantially generated when said polynucleotide drug is not hybridized to said target; and

(b) forming a hybrid comprising said polynucleotide drug and said target polynucleotide.

44. The method of Claim 59 wherein said characteristic is the ability of said entities to intercalate into said hybrid.

45. The method of Claims 60 wherein said entities are aromatic dyes.

46. A compound comprising at least one moiety having the structure



wherein B represents a base selected from the group consisting of pyrimidines, purines, and deazapurines, provided that whenever B is a pyrimidine, the sugar is attached to the N¹-position of the pyrimidine, and whenever B is a purine or deazapurine, the sugar is attached to the N⁹-position of the purine or deazapurine;

wherein "Phen" represents any phenanthridine moiety;

wherein said L.A. is a linker arm comprising at least three carbon atoms, and is attached to the 5-position of said phenanthridine moiety; and

wherein z is H or O-.

47. The compound of Claim 46 wherein said purine is adenine, or guanine, and wherein said linker arm is attached at the 8 or exocyclic 6-amino position when said purine is adenine, or at the 8-position when said purine is guanine.
48. The compound of Claim 47 wherein said linker arm is attached at the 8-position.
49. The compound of Claim 46 wherein said pyrimidine is uracil or cytosine, and wherein said linker arm is attached at the 5 or 6 position when said pyrimidine is uracil, or at the 5,6 or exocyclic 4-amino position when said pyrimidine is cytosine..
50. The compound of Claim 49 wherein said linker arm is attached at the 5-position.
51. The compound of Claim 46 wherein base is deazapurine and said linker arm is attached to the 7 or 8 position of said deazapurine. "
52. The compound of Claim 46 wherein said linker arm is covalently attached to said phenanthridine moiety.
53. The compound of Claim 46 wherein said linker arm

is non-covalently attached to said phenanthridine moiety.

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